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Title: OPTIMIZATION OF BIOFUEL PRODUCTION FROM TRANSGENIC MICROALGAE
Grant/Contract Number: FA9550-07-1-0169
Reporting Period: August 30 2007 to August 31, 2008

Chlorella transformation: We have developed nuclear transformation vectors based on the Ble resistance gene. We observed that Chlorella was resistant to a number of antibiotics that other algae were sensitive too. This necessitated a screen for the best antibiotic resistance genes to use for transformation selectable markers. Using the Chlamydomonas ss-rbcl and psaD promoters we have obtained transgenic Chlorella cells at high frequency that have been confirmed by PCR analysis. We also have now completed the genome sequence of the actin gene and its promoter from C. prothecoides. This promoter is now being incorporated into a transformation vector.

Optimization of light harvesting antennae size: Typically, wild type algae light saturate at 1/3 of full sunlight intensity. This is associated with an over-efficient light harvesting chlorophyll a/b (LHC) complexes that light saturate the reaction centers. By reducing the antennae size it has been shown in the lab that it is possible to increase photosynthetic quantum efficiency.

Using two independent strategies we have successfully altered the chlorophyll a/b ratio, and as demonstrated by chlorophyll fluorescence induction kinetics, the LHC antennae size of transgenic algae expressing either a chlorophyll a oxidase RNAi construct or by over-expressing chlorophyll b reductase. Wild type algae have chlorophyll a/b ratios of 2. The transgenics have chlorophyll a/b ratios approaching 4 indicative of a 90% reduction in chlorophyll b content. We are currently planning to do high light (full sunlight) growth experiments to determine empirically the best LHC content for optimal growth at high light intensities.

Non-destructive oil extraction: Harvesting algae and extracting oil accounts for 50% of the cost of producing algal biofuels. Much of this cost is associated with concentrating algae from 0.1% of the mass of the pond to nearly 80% of the extractable material. This dewatering process is energetically very expensive. Also, algae are currently destroyed during the extraction process so a new culure must be grown each time the algae are harvested. A more efficient oil extraction technology would eliminate or substantially reduce the dewatering and non-destructively extract oils so the cultures would not need to be re-grown. The biocompatible solvent system we have developed meets these criteria. Using a variety for short, straight-chain alkanes and optimal mixing procedures we have been able to extract quantitatively neutral lipids from Chlorella and Nanochloropsis. We have gone as many as five extraction cycles with nanochloropsis with no apparent reduction in growth. We have submitted a patent on this process.

Metabolic Engineering: We are focusing on engineering both lipid synthesis and photosynthetic carbon fixation to optimize oil production. Gene constructs have been developed to engineer pyruvate metabolism in algae and are ready to transform. We are nearing completion of a Rubisco construct linked to carbonic anhydrase to increase the CO2 concentration near the active site of Rubisco to inhibit photorespiration. In addition, we have started a project on characterizing the proteomes of cells induced to produce oils under different conditions. These studies are expected to provide insights into which genes to target for enhanced oil production.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
	FINAL REPORT	01 FEB 07 – 31 MAY 08

4. TITLE AND SUBTITLE
OPTIMIZATION OF BIOFUEL PRODUCTION FROM TRANSGENIC MICROALGAE

5a. CONTRACT NUMBER

5b. GRANT NUMBER
FA9550-07-1-0169

5c. PROGRAM ELEMENT NUMBER
61102F

6. AUTHOR(S)
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5d. PROJECT NUMBER
2312

5e. TASK NUMBER
AX

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
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Suite 325, Room 3112
Arlington, VA 22203-1768

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-OSR-VA-TR-2012-0501

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approve for Public Release

13. SUPPLEMENTARY NOTES

14. ABSTRACT – We have made excellent progress on the development of genetic tools for the transformation of the microalga, chlorella protothecoides. We have developed nuclear transformation vectors and identified both heterologous and homologous gene promters to drive transgene expression. We have demonstrated a range in reduction in the light harvesting chlorophyll a/b binding protein content in transgenic Chlamydomonas (as a model system before the C. protothecoides vectors were developed) using both cholorophyll a oxidase RNAi constructs and by overexpression of chlorophyll b reductase. These transgenic algae (with different ntennae sizes) are now being tested in growth experiments at high light intensities to determine the optimal antennae size for biomass accumulation. We also continue to make excellent progress on the nondestructive lipid extraction process which substantially increases oil production while reducing harvesting costs. Finally, we are now well positioned to metabolically engineer Chlorella lipid biosynthesis and photosynthetic electron transfer pathways to optimize oil production. This and an analysis of the proteomics of oil production are the focus areas for the coming year.

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a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(Include area code)*

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